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 FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 18:49:53 ON 17 AUG 2004
L1         96979 S (FUSION OR CHIMER?) (S) (PROTEIN OR CONSTRUCT)
L2         86807 S HORMONE (S) RECEPTOR
L3         23673 S CYTOKINE (S) RECEPTOR
L4        313064 S "CELL PROLIFERATION" OR (PROLIFERATION (S) CELL)
L5         24881 S "G-CSF" OR GCSF
L6         15901 S OZAWA?/AU OR IBARAKI?/AU
L7             2 S L6 AND L2 AND L3
L8             2 DUP REM L7 (0 DUPLICATES REMOVED)
L9             2 S L1 AND L2 AND L3 AND L4
L10        71439 S ESTROGEN (S) RECEPTOR
L11          241 S L10 AND L3
L12           46 S L11 AND L4
L13           18 S L12 NOT PY>=2000
L14           15 DUP REM L13 (3 DUPLICATES REMOVED)
L15        10927 S "EXOGENOUS GENE" OR "TARGET GENE"
L16        16480 S "MULTIPLE VECTOR" OR "DUAL VECTOR" OR "CO-TRANSFECTION" OR "C
L17          241 S L15 AND L16
L18            0 S L17 AND L2 AND L3
L19           26 S L17 AND L2
L20            7 S L19 AND "BINDING DOMAIN"
L21            3 DUP REM L20 (4 DUPLICATES REMOVED)
L22           17 S L17 AND L4
L23           12 DUP REM L22 (5 DUPLICATES REMOVED)
L24            0 S L17 AND L5
L25            6 S L17 AND L6
L26            2 DUP REM L25 (4 DUPLICATES REMOVED)
L27         6250 S "LIGAND BINDING DOMAIN"
L28         1053 S L27 (P) L2
L29         2451 S L3 (P) L4
L30            2 S L28 AND L29
L31            1 DUP REM L30 (1 DUPLICATE REMOVED)
L32          531 S L1 AND L27
L33           99 S L32 AND L28
L34           63 S L33 NOT PY>=2000
L35           34 DUP REM L34 (29 DUPLICATES REMOVED)
L36          109 S L6 AND L1
L37            6 S L36 AND L27
L38            2 DUP REM L37 (4 DUPLICATES REMOVED)

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L Number	Hits	Search Text	DB	Time stamp
1	15481	IL NEAR2 ("1" or "2" or "3" or "4" or "5" or "6" or "9")	USPAT	2004/08/17 16:45
2	5560506	vector or vector system or cloning system or expression constructs	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
3	1036727	cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
4	4916	"hematopoietic stem cells"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
5	1173487	ligand binding domain or protein binding domain or selective proliferation	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
6	209868	steroid hormone receptor	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
7	3845	(vector or vector system or cloning system or expression constructs) and (cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells" and (ligand binding domain or protein binding domain or selective proliferation) and (steroid hormone receptor)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
8	158972	((vector or vector system or cloning system or expression constructs) and (cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells" and (ligand binding domain or protein binding domain or selective proliferation) and (steroid hormone receptor)) and cytokine receptor	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
9	3671	"ligand binding domain"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
11	116	("ligand binding domain" same "steroid hormone receptor") and (cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
13	788459	"fusion protein" same proliferat? activity	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
14	11425	"fusion protein" same (proliferat? activity)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
16	2986	((vector or vector system or cloning system or expression constructs) same ("fusion protein" same proliferat? activity)) and "ligand binding domain"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
18	195	((((vector or vector system or cloning system or expression constructs) same ("fusion protein" same proliferat? activity)) and "ligand binding domain") and "hematopoietic stem cells") and "hematopoietic stem cells"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46

19	195	((((vector or vector system or cloning system or expression constructs) same ("fusion protein" same proliferat? activity)) and "ligand binding domain") and "hematopoietic stem cells") and (cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:46
21	5547	"chimeric protein"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
22	4343	"ligand binding domain" or "signal transduction domain" or "protein binding domain"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
25	2116	(cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells"	USPAT	2004/08/17 16:47
26	1861	((cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells") and (cytoplasmic domain same ligand binding domain)	USPAT	2004/08/17 16:47
27	1587	(((cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells") and (cytoplasmic domain same ligand binding domain)) and (G-CSF or estrogen receptor)	USPAT	2004/08/17 16:47
28	1509	(((cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells") and (cytoplasmic domain same ligand binding domain)) and (G-CSF and estrogen receptor)	USPAT	2004/08/17 16:47
29	1583	(((cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells") and (cytoplasmic domain same ligand binding domain)) and (G-CSF receptor and estrogen receptor)	USPAT	2004/08/17 16:47
32	0	((((cell proliferation and (G-CSF or estrogen receptor or c-Abl or tyrosine kinase)) and "hematopoietic stem cells") and (cytoplasmic domain same ligand binding domain)) and (G-CSF receptor and estrogen receptor)) and "estrogen receptor ligand binding domain"	USPAT	2004/08/17 16:47
34	328600	vector or plasmid	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
35	50781	(fusion or chimer$) WITH protein	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
36	253	"ligand binding domain" WITH steroid	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
37	12539	cytokine WITH receptor	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
38	59230	"cell proliferation" or (proliferation WITH cell)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
40	7113	"hormone receptor"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47

41	2456	"cytokine receptor"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
42	36197	"cell proliferation"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
43	54289	cell WITH proliferat$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
44	42446	(fusion or chimer$) NEAR3 (protein or construct)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
45	21798	"binding domain"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
46	11511	G-CSF or (granulocyte WITH stimulat$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
47	5254	estrogen NEAR3 receptor	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
48	107255	ozawa.in. or itoh.in. or sakata.in. or hasegawa.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
50	121	"hormone receptor" SAME "cytokine receptor"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
52	53	("hormone receptor" SAME "cytokine receptor") and "cell proliferation"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
53	53	(("hormone receptor" SAME "cytokine receptor") and "cell proliferation") and (cell WITH proliferat$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
54	40	((("hormone receptor" SAME "cytokine receptor") and "cell proliferation") and (cell WITH proliferat$)) and ((fusion or chimer$) NEAR3 (protein or construct))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
56	17299	(stem or hematopoietic) NEAR2 cell	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
10	117	"ligand binding domain" same "steroid hormone receptor"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
12	7	("ligand binding domain" same "steroid hormone receptor") and "hematopoietic stem cells"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
15	49	("fusion protein" same (proliferat? activity)) and (("ligand binding domain" same "steroid hormone receptor") and (cell proliferation and (G-CSF or estrogen receptor or c-Ab1 or tyrosine kinase)))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
17	195	(((vector or vector system or cloning system or expression constructs) same ("fusion protein" same proliferat? activity)) and "ligand binding domain") and "hematopoietic stem cells"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47

20	195	((((vector or vector system or cloning system or expression constructs) same ("fusion protein" same proliferat? activity)) and "ligand binding domain") and "hematopoietic stem cells") and (cell proliferation and (G-CSF or estrogen receptor or c-Ab1 or tyrosine kinase)) and "hematopoietic stem cells"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:47
23	619	"chimeric protein" and ("ligand binding domain" or "signal transduction domain" or "protein binding domain")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:48
24	281	("chimeric protein" and ("ligand binding domain" or "signal transduction domain" or "protein binding domain")) and "cell proliferation"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:48
30	1	"stimulating factor" with "cytoplasmic domain"	USPAT	2004/08/17 16:48
31	14	"stimulating factor" same "cytoplasmic domain"	USPAT	2004/08/17 16:48
33	1564	((((cell proliferation and (G-CSF or estrogen receptor or c-Ab1 or tyrosine kinase)) and "hematopoietic stem cells") and (cytoplasmic domain same ligand binding domain)) and (G-CSF receptor and estrogen receptor)) and (estrogen receptor with ligand binding domain)	USPAT	2004/08/17 16:48
39	14	(vector or plasmid) and ((fusion or chimer$) WITH protein) and ("ligand binding domain" WITH steroid) and (cytokine WITH receptor) and ("cell proliferation" or (proliferation WITH cell))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:48
49	3	(ozawa.in. or itoh.in. or sakata.in. or hasegawa.in.) and "hormone receptor" and "cytokine receptor"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:48
51	1	("hormone receptor" SAME "cytokine receptor") SAME "cell proliferation"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:49
55	34	((((("hormone receptor" SAME "cytokine receptor") and "cell proliferation") and (cell WITH proliferat$)) and ((fusion or chimer$) NEAR3 (protein or construct))) and "binding domain"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:49
57	26	(("hormone receptor" SAME "cytokine receptor") and "cell proliferation") and ((stem or hematopoietic) NEAR2 cell)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:49
58	2	5686281.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:49
59	13608	(IL NEAR2 ("1" or "2" or "3" or "4" or "5" or "6" or "9")) and (cell proliferation and (G-CSF or estrogen receptor or c-Ab1 or tyrosine kinase))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:50
60	121	("hormone receptor" SAME "cytokine receptor") and (steroid hormone receptor)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:50
62	2	((("hormone receptor" SAME "cytokine receptor") and (steroid hormone receptor)) and ("fusion protein" same proliferat? activity)) and ((((vector or vector system or cloning system or expression constructs) same ("fusion protein" same proliferat? activity)) and "ligand binding domain") and "hematopoietic stem cells")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:51

61	105	(("hormone receptor" SAME "cytokine receptor") and (steroid hormone receptor)) and ("fusion protein" same proliferat? activity)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:59
63	105	((("hormone receptor" SAME "cytokine receptor") and (steroid hormone receptor)) and ("fusion protein" same proliferat? activity)) and (steroid hormone receptor)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 16:59
64	105	(((("hormone receptor" SAME "cytokine receptor") and (steroid hormone receptor)) and ("fusion protein" same proliferat? activity)) and (cytokine WITH receptor)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:11
65	2	5747292.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:17
66	2	6416998.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:18
67	0	6416998.pn. and "cytokine receptor"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:18
68	0	6416998.pn. and "proliferation domain"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:19
69	2	5837544.pn.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:19
70	1	5837544.pn. and cytokine	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:19
71	117	("ligand binding domain" same "steroid hormone receptor") and steroid	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:20
72	1	(5837544.pn. and cytokine) and steroid	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:50
73	6737	"dual vector" or "binary vector" or "multiple vector" or "cotransformation" or "cotransfection"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:51
74	101	("dual vector" or "binary vector" or "multiple vector" or "cotransformation" or "cotransfection") and (steroid hormone receptor) and "cytokine receptor"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 17:52
75	92	(("dual vector" or "binary vector" or "multiple vector" or "cotransformation" or "cotransfection") and (steroid hormone receptor) and "cytokine receptor") and ((fusion or chimer$) WITH protein)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 18:00
76	92	((("dual vector" or "binary vector" or "multiple vector" or "cotransformation" or "cotransfection") and (steroid hormone receptor) and "cytokine receptor") and ((fusion or chimer$) WITH protein)) and (ligand binding domain or protein binding domain or selective proliferation)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 18:00
77	16717	"exogenous gene" or "target gene"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 18:01

78	23	((((("dual vector" or "binary vector" or "multiple vector" or "cotransformation" or "cotransfection") and (steroid hormone receptor) and "cytokine receptor") and ((fusion or chimer$) WITH protein)) and (ligand binding domain or protein binding domain or selective proliferation)) and ("exogenous gene" or "target gene")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/17 18:01

ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1999031206 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9813663
TITLE: Apoptosis-mediated regulation of recombinant human granulocyte colony-stimulating factor production by genetically engineered fibroblasts.
AUTHOR: Kokubun M; Kume A; Urabe M; Mano H; Okubo M; Kasukawa R; Kakizuka A; **Ozawa K**
CORPORATE SOURCE: Division of Genetic Therapeutics, Jichi Medical School, Tochigi, Japan.
SOURCE: Gene therapy, (1998 Jul) 5 (7) 923-9.
Journal code: 9421525. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981130

AB We investigated the feasibility of an inducible apoptosis system to regulate cells genetically engineered for ectopic cytokine production. In a previous study, cDNA encoding the **ligand-binding domain** of the rat estrogen receptor was fused to the sequence for murine Fas transmembrane and cytoplasmic regions, and expression of the **fusion protein** (MfasER) in L929 fibroblasts resulted in estrogen-dependent apoptosis. We applied this MfasER/estrogen strategy to apoptosis-mediated regulation of cytokine production, using the human granulocyte colony-stimulating factor (G-CSF) as a model. Upon estrogen treatment, the G-CSF producers expressing MfasER showed an apoptotic phenotype and died in several hours, with termination of G-CSF production. This estrogen-induced apoptosis was not influenced by whether the target cells were proliferating or resting, unlike a conventional suicide system involving the herpes simplex virus 1 thymidine kinase (HSVtk). That is, estrogen induced prompt and extensive apoptosis in the resting cells which expressed MfasER, while ganciclovir treatment induced only partial reduction of the resting cells which expressed HSVtk. These results imply the feasibility of apoptosis-mediated regulation of cytokine production by genetically modified cells for supplement gene therapy.

L38 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998409361 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9738981
TITLE: Fas and mutant estrogen receptor chimeric gene: a novel suicide vector for tamoxifen-inducible apoptosis.
AUTHOR: Kodaira H; Kume A; Ogasawara Y; Urabe M; Kitano K; Kakizuka A; **Ozawa K**
CORPORATE SOURCE: Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical School, Tochigi.
SOURCE: Japanese journal of cancer research : Gann, (1998 Jul) 89 (7) 741-7.
Journal code: 8509412. ISSN: 0910-5050.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980924

AB Several cancer gene therapy strategies involve suicide genes to kill the neoplasm, or to regulate effector cells such as lymphocytes. We have developed an inducible apoptosis system with a Fas-estrogen receptor

fusion protein (MfasER) for rapid elimination of transduced cells. In the present study, we further improved this molecular switch for estrogen-inducible apoptosis to overcome concerns with the wild-type estrogen receptor and its natural ligand, 17beta-estradiol (E2). The **ligand-binding domain** of MfasER was replaced with that of a mutant estrogen receptor which is unable to bind estrogen yet retains affinity for a synthetic ligand, 4-hydroxytamoxifen (Tm). The resultant **fusion protein** (MfasTmR) and MfasER were expressed in L929 cells for examination of their ligand specificities. Tm induced apoptosis in MfasTmR-expressing cells (L929MfasTmR) at 10(-8) M or higher concentrations, but induced no apoptosis in MfasER-expressing cells (L929MfasER) at up to 10(-6) M. On the other hand, E2 induced apoptosis in L929MfasER at concentrations as low as 10(-10)-10(-9) M, while it did so partially in L929MfasTmR at concentrations greater than 10(-7) M. Thus, L929MfasTmR cells were highly susceptible to Tm, but refractory to E2, with 100-1,000 times more tolerance than L929MfasER. These results suggest that the MfasTmR/Tm system would induce apoptosis in the target cells more safely in vivo, working independently of endogenous estrogen.

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ANSWER 1 OF 15 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
ACCESSION NUMBER: 1999424974 EMBASE
TITLE: Postmenopausal hormone replacement therapy and the vascular wall: Mechanisms of 17 β-estradiol's effects on vascular biology.
AUTHOR: Joswig M.; Hach-Wunderle V.; Ziegler R.; Nawroth P.P.
CORPORATE SOURCE: Dr. M. Joswig, Dept. Internal Medicine I, Endocrinology and Metabolism, University of Heidelberg, Bergheimer Strasse 58, D-69115 Heidelberg, Germany
SOURCE: Experimental and Clinical Endocrinology and Diabetes, (1999) 107/8 (477-487).
Refs: 101
ISSN: 0947-7349 CODEN: ECEDFQ
COUNTRY: Germany
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 003 Endocrinology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB 17 β-estradiol (E2) protects against atherosclerosis independent of changes in plasma lipoproteins in a variety of animal models, which is explained by direct effects of E2 on the vascular wall. E2 improves vasomotion by modulation of vasoconstrictor and vasodilator systems through endothelium-dependent and endothelium-independent mechanisms. E2 affects the remodeling of the vascular wall by inhibiting smooth muscle **cell proliferation** and accelerating reendothelialization of injured blood vessels. E2 modulates the vascular inflammatory response by inhibiting **cytokine** production, **cytokine**-induced expression of **cell** adhesion molecules and platelet aggregation/adhesion. This review focuses on the cellular and molecular mechanisms underlying these vasculoprotective actions of E2. E2 can act through nongenomic stimulation of membrane/intracellular mediators and/or the classical genomic pathway of steroid actions, which is dependent on transcription and protein synthesis. The existence of at least two nuclear **estrogen receptor** (ER) subtypes α and β and a putative membrane ER present the potential of tissue-specific as well as biologically different E2 actions. Nuclear ERs act as ligand-activated transcription factors and can affect gene regulation by interaction with the classical **estrogen** response element or other nonreceptor transcription factors. The molecular basis of genomic E2 actions by identifying transcription factors and regulatory elements involved in the induction and inhibition of E2 regulated gene expression is only at the beginning of being understood. The impact of E2-mediated increased NO availability on the hemodynamic and antiatherosclerotic actions of E2 is still a debate of controversy.

L14 ANSWER 2 OF 15 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000067554 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10599447
TITLE: Complexity, retinoid-responsive gene networks, and bladder carcinogenesis.
AUTHOR: Hurst R E; Waliszewski P; Waliszewska M; Bonner R B; Benbrook D M; Dar A; Hemstreet G P 3rd
CORPORATE SOURCE: Department of Urology, University of Oklahoma Health Sciences Center, Oklahoma City 73190, USA.
SOURCE: Advances in experimental medicine and biology, (1999) 462 449-67. Ref: 92
Journal code: 0121103. ISSN: 0065-2598.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000107

AB Carcinogenesis involves inactivation or subversion of the normal controls of proliferation, differentiation, and apoptosis. However, these controls are robust, redundant, and interlinked at the gene expression levels, regulation of mRNA lifetimes, transcription, and recycling of proteins. One of the central systems of control of proliferation, differentiation and apoptosis is retinoid signaling. The hRAR alpha nuclear receptor occupies a central position with respect to induction of gene transcription in that when bound to appropriate retinoid ligands, its homodimers and heterodimers with hRXR alpha regulate the transcription of a number of retinoid-responsive genes. These include genes in other signaling pathways, so that the whole forms a complex network. In this study we showed that simple, cause-effect interpretations in terms of hRAR alpha gene transcription being the central regulatory event would not describe the retinoid-responsive gene network. A set of cultured bladder-derived cells representing different stages of bladder tumorigenesis formed a model system. It consisted of 2 immortalized bladder cell lines (HUC-BC and HUC-PC), one squamous cell carcinoma cell line (SCaBER), one papilloma line (RT4), and 4 transitional cell carcinomas (TCC-Sup, 5637, T24, J82) of varying stages and grades. This set of cells were used to model the range of behaviors of bladder cancers. Relative gene expression before (constitutive) and after treatment with 10 microM all-trans-retinoic acid (aTRA) was measured for androgen and **estrogen receptor**; a set of genes involved with retinoid metabolism and action, hRAR alpha nd beta, hRXR alpha and beta CRBP, CRABP I and II; and for signaling genes that are known to be sensitive to retinoic acid, EGFR, **cytokine** MK, ICAM I and transglutaminase. The phenotype for inhibition of proliferation and for apoptotic response to both aTRA and the synthetic retinoid 4-HPR was determined. Transfection with a CAT-containing plasmid containing an aTRA-sensitive promoter was used to determine if the common retinoic acid responsive element (RARE)-dependent pathway for retinoid regulation of gene expression was active. Each of the genes selected is known from previous studies to react to aTRA in a certain way, either by up- or down-regulation of the message and protein. A complex data set not readily interpretable by simple cause and effect was observed. While all cell lines expressed high levels of the mRNAs for hRXR alpha and beta that were not altered by treatment with exogenous aTRA, constitutive and stimulated responses of the other genes varied widely among the cell lines. For example, CRABP I was not expressed by J82, T24, 5637 and RT4, but was expressed at low levels that did not change in SCaBER and at moderate levels that decreased, increased, or decreased sharply in HUC-BC, TCC-Sup and HUC-PC, respectively. The expression of hRAR alpha, which governs the expression of many retinoid-sensitive genes, was expressed at moderate to high levels in all cell lines, but in some it was sharply upregulated (TCC-Sup, HUC-PC and J82), remained constant (5637 and HUC-BC), or was down-regulated (SCaBER, T24 and RT4). The phenotypes for inhibition of **proliferation** showed no obvious relationship to the expression of any single gene, but **cell** lines that were inhibited by aTRA (HUC-BC and TCC-Sup) were not sensitive to 4-HPR, and vice versa. One line (RT4) was insensitive to either retinoid. Transfection showed very little retinoid-stimulated transfection of the CAT reporter gene with RT4 or HUC-PC. About 2-fold enhancement transactivation was observed with SCaBER, HUC-BC, J82 and T24 cells and 3-8 fold with 5637, TCC-Sup cells. In HUC-BC, a G to T point mutation was found at position 606 of the hRAR alpha gene. This mutation would substitute tyrosine for asparagine in a highly conserved domain. These data indicate that retinoid signaling is probably a frequent target of

inactivation in bladder carcinogenesis. (ABSTRAC

L14 ANSWER 3 OF 15 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1999141384 EMBASE
TITLE: In vitro test systems for the evaluation of the estrogenic activity of natural products.
AUTHOR: Diel P.; Smolnikar K.; Michna H.
CORPORATE SOURCE: Dr. P. Diel, Inst. fur Experimentelle Morphologie, Deutsche Sporthochschule Koln, Carl Diem-Weg 6, D-50933 Koln, Germany. diel@hrz.dshs-koeln.de
SOURCE: Planta Medica, (1999) 65/3 (197-203).
Refs: 55
ISSN: 0032-0943 CODEN: PLMEAA
COUNTRY: Germany
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Many compounds of plant origin with the ability to bind to the **estrogen receptor** have been identified in the last decades. There is evidence that the consumption of some of these phytoestrogens may have beneficial effects but it also seems possible that others may act as endocrine disrupters. For this reason there is a need to characterise the estrogenic potency of these substances. In vitro test systems offer the possibility to screen compounds very efficiently. Routinely in use and widespread for the determination of estrogenicity are: (I) **receptor** binding assays, (II) **cell-proliferation** assays (E-screens), (III) reporter gene assays, and (IV) the analysis of the regulation of endogenous **estrogen** sensitive genes in **cell** lines. The basis of all these test systems are molecular mechanisms which are involved in the classical **estrogen** action. In addition, in the last years several test systems for the investigation of non-classical estrogenic effects have been established. An example for such an effect is the modulation of the expression of interleukin-6, a **cytokine** that appears to be a key molecule in the osteoporotic process, by estrogens. Summarising the advantages and the issues of all presented in vitro test systems, it seems to be evident that only the analysis of results obtained in a combination of several in vitro test systems may validly predict effects in vivo.

L14 ANSWER 4 OF 15 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998323536 EMBASE
TITLE: Interleukin 4 inhibits growth and induces apoptosis in human breast cancer cells.
AUTHOR: Gooch J.L.; Lee A.V.; Yee D.
CORPORATE SOURCE: D. Yee, Department of Medicine, Division of Medical Oncology, Univ. of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284-7884, United States. doug@oncology.uthscsa.edu
SOURCE: Cancer Research, (15 Sep 1998) 58/18 (4199-4205).
Refs: 42
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Interleukin-4 (IL-4) is a pleiotropic **cytokine** produced by mast

cells and T lymphocytes that promotes **proliferation** and immunoglobulin class- switching in B cells. IL-4 receptors (IL-4Rs) are also expressed by nonhematopoietic cells as well as some tumor cells. Unlike its mitogenic effect on B cells, IL-4 inhibits the growth of some cancer cells in vitro. In this study, we show that IL-4R is expressed by breast and ovarian cancer **cell** lines. Furthermore, anchorage-dependent and -independent growth of breast cancer **cell** lines MCF-7 and MDA-MB-231 is inhibited by IL-4 treatment, and this effect requires IL-4R. Interestingly, IL-4 only inhibited proliferating breast cancer cells and had no effect on basal, unstimulated growth. We therefore characterized the effect of IL-4 on breast cancer **cell** growth stimulated by either estradiol or insulin-like growth factor I (IGF-I). In both anchorage-dependent and -independent growth assays, IL-4 inhibited estradiol-stimulated growth. The antiestrogen effect of IL-4 was not due to IL-4 interference with the **estrogen receptor**, because IL-4 did not interfere with **estrogen receptor** -mediated reporter gene transactivation. In contrast, IL-4 had no effect on IGF-I-stimulated **proliferation**. Because IGF-I is known to inhibit programmed **cell** death, we examined apoptosis as a possible mechanism of IL-4 action. We established that IL-4 induced apoptosis in breast cancer cells by five independent criteria: (a) morphological indicators including pyknotic nuclei and cytoplasmic condensation; (b) DNA fragmentation; (c) the formation of DNA laddering; (d) the cleavage of poly(ADP-ribose) polymerase; and (e) the presence of cells with sub-G1 DNA content. IL-4 increased the percentage of apoptotic cells in MCF-7 and MDA- MB-231 cells 6.0- and 6.7-fold over that of the control, respectively. Finally, the addition of IGF-I reversed IL-4-induced apoptosis, suggesting that the mechanism of IL-4-induced growth inhibition in human breast cancer cells is the induction of programmed **cell** death.

L14 ANSWER 5 OF 15 EMBASE
on STN

ACCESSION NUMBER: 1998414012 EMBASE

TITLE: Differential abilities of activated Raf oncoproteins to abrogate cytokine dependency, prevent apoptosis and induce autocrine growth factor synthesis in human hematopoietic cells.

AUTHOR: McCubrey J.A.; Steelman L.S.; Hoyle P.E.; Blalock W.L.; Weinstein-Oppenheimer C.; Franklin R.A.; Cherwinski H.; Bosch E.; McMahon M.

CORPORATE SOURCE: J.A. McCubrey, Department Microbiology Immunology, East Carolina Univ. School Medicine, Brody Building 5N98C, Greenville, NC 27858, United States

SOURCE: Leukemia, (1998) 12/12 (1903-1929).
Refs: 124
ISSN: 0887-6924 CODEN: LEUKED

COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Raf is a key serine-threonine protein kinase which participates in the transmission of growth, anti-apoptotic and differentiation messages. These signals can be initiated after **receptor** ligation and are transmitted to members of the MAP kinase cascade that subsequently activate transcription factors controlling gene expression. Raf is a member of a multigene family which includes: Raf-1, A-Raf and B-Raf. The roles that individual Raf kinases play in the regulation of normal and malignant hematopoietic **cell** growth are not clear. The following studies show that all three Raf kinases are functionally present in certain human hematopoietic cells, and their aberrant expression can

result in abrogation of **cytokine** dependency. **Cytokine** -dependent TF-1 cells were infected with retroviruses encoding amino-terminal deleted (Δ) A-Raf, B-Raf and Raf-1 proteins. These Raf proteins were conditionally inducible as they were fused to the hormone-binding domain of the **estrogen receptor** (ER). A hierarchy in the abilities of Raf-containing retroviruses to abrogate **cytokine** dependency was observed as ΔA-Raf:ER was 20- to 200-fold more efficient than either ΔRaf-1:ER or ΔB-Raf:ER, respectively. This result was unexpected as A-Raf is an intrinsically weaker kinase than either Raf-1 or B-Raf. The activated Raf proteins induced downstream MEK and MAP (ERK1 and ERK2) kinase activities in the cells which proliferated in response to Raf activation. Furthermore, a functional MEK signaling pathway was necessary as treatment of the cells with a MEK1-inhibitor suppressed Raf-mediated **proliferation**. To determine whether the regulatory phosphorylation residues contained in the modified Raf oncoproteins were necessary for transformation, they were altered by site-directed mutagenesis. Substitution of the regulatory phosphorylation tyrosine residues with phenylalanine in either A-Raf or Raf-1 reduced the capacity of these oncoproteins to abrogate **cytokine** dependency. In contrast, changing the critical aspartic acid residues of B-Raf to either tyrosine or phenylalanine increased the frequency of estradiol-responsive cells. Thus, the amino acids present in the regulatory residues modulated the capability of Raf proteins to abrogate the **cytokine** dependency of TF-1 cells. Differences in the levels of Raf and downstream kinase activities were observed between **cytokine**-dependent and estradiol-responsive ΔRaf:ER-infected cells as estradiol-responsive cells usually expressed more Raf and MEK activity than GM-CSF-dependent, ΔRaf:ER-infected cells. Abrogation of **cytokine** dependency by the activated ΔRaf:ER proteins was associated with autocrine growth factor synthesis which was sufficient to promote the growth of uninfected TF-1 cells. In summary, these observations indicate that the aberrant expression of certain activated ΔRaf:ER oncoproteins can alter the **cytokine** dependency of human hematopoietic TF-1 cells. These cells will be useful in evaluating the roles of the individual Raf oncoproteins in signal transduction, **cell** cycle progression, autocrine transformation, regulation of apoptosis and differentiation. Moreover, these Raf-infected cells may be important in evaluating the efficacy of novel anticancer drugs designed to inhibit Raf and downstream signal transduction molecules.

L14 ANSWER 6 OF 15 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:305398 BIOSIS
DOCUMENT NUMBER: PREV199800305398
TITLE: Cell density and oestrogen both stimulate alpha2-macroglobulin receptor expression in breast cancer cell T-47D.
AUTHOR(S): Li, Yonghe; Wood, Nick [Reprint author]; Donnelly, Peter; Yellowlees, David
CORPORATE SOURCE: Dep. Surg., Univ. Queensl., North Queensl. Clin. Sch., PO Box 1805, Townsville, Qld. 4810, Australia
SOURCE: Anticancer Research, (March-April, 1998) Vol. 18, No. 2A, pp. 1197-1202. print.
CODEN: ANTRD4. ISSN: 0250-7005.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Jul 1998
Last Updated on STN: 15 Jul 1998

AB Background: Oestrogen is an important hormone supporting the growth and evolution of breast cancer. alpha2-Macroglobulin **receptor**/low density lipoprotein **receptor**-related protein (alpha2MR/LRP) is a multifunctional membrane **receptor** for endocytosis of various ligands involved in protease and **cytokine** activity regulation. The effects of oestrogen on the level of expression of this receptor may

be important in breast tumour progression. Materials and Methods: T-47D breast cancer cells were grown in media with controlled oestrogen levels, and flow cytometry and Western blotting were used to compare their alpha2MR/LRP expression levels. Results: Addition of oestrogen to T-47D cells caused a marked increase in alpha2MR/LRP expression, coinciding with a tripling of **cell proliferation**. T-47D cells at high cell culture densities had similarly raised alpha2MR/LRP expression levels. Conclusions: Regulation of alpha2MR/LRP expression in the oestrogen receptor-positive breast cancer cell line T-47D can be effected by both cell culture density alone and by oestrogen.

L14 ANSWER 7 OF 15 EMBASE
on STN

ACCESSION NUMBER: 1998031696 EMBASE
TITLE: Differential regulation of the human 'leukemia inhibitory factor' (LIF) promoter in T47D and MDA-MB 231 breast cancer cells.
AUTHOR: Bamberger A.-M.; Thuneke I.; Schulte H.M.
CORPORATE SOURCE: Dr. A.-M. Bamberger, IHF, Institute Hormone Fertility Research, University of Hamburg, Grandweg 64, 22529 Hamburg, Germany
SOURCE: Breast Cancer Research and Treatment, (1998) 47/2 (153-161).
Refs: 27
ISSN: 0167-6806 CODEN: BCTRD6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
016 Cancer
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Leukemia inhibitory factor (LIF) is a pleiotropic inflammatory **cytokine**. A potential role for LIF in the pathogenesis of human breast cancer was recently indicated by the finding that LIF is produced by MDA-MB 231 breast cancer cells and that it stimulates **proliferation** of the T47D and MCF-7 breast cancer **cell** lines. Despite its role as a possible therapeutic target in breast cancer, the transcriptional regulation of the LIF gene in breast cancer cells has not been investigated so far. In this context, we investigated the regulation of the human LIF promoter (human LIF666-luciferase) by ovarian steroids in transient transfection assays in MDA-MB 231 and T47D cells. Since the MDA-MB 231 cells are devoid of both **estrogen** (ER) and progesterone (PR) receptors, these cells were co-transfected with the respective **receptor** expression vector. Estradiol induced no stimulation in either T47D or ER-transfected MDA-MB 231 cells. Treatment with the progesterone agonist MPA (medroxy-progesterone acetate) resulted in induction of LIF transcription in PR-transfectant MDA-MB 231 cells, while it had no effect in T47D cells. Both PR isoforms (PR-B and PR-A) were effective in inducing the LIF promoter in MDA-MB 231 cells, and this effect was inhibited by the progestin antagonist RU 486. The stimulatory effect of MPA was maintained on deletion constructs (h274LIF-Luc, h148LIF-Luc and h82LIF-Luc), indicating that 82 bp are sufficient to mediate this effect. Our results indicate that the LIF promoter is transcriptionally active in human breast cancer cells and its activity can be modulated by progestins and anti-progestins in cells expressing the LIF protein, which might have therapeutic implications.

L14 ANSWER 8 OF 15 EMBASE
on STN

ACCESSION NUMBER: 97284642 EMBASE
DOCUMENT NUMBER: 1997284642
TITLE: Evaluation of the major metabolites of raloxifene as modulators of tissue selectivity.

AUTHOR: Dodge J.A.; Lugar C.W.; Cho S.; Short L.L.; Sato M.; Yang N.N.; Spangel L.A.; Martin M.J.; Phillips D.L.; Glasebrook A.L.; Osborne J.J.; Frolik C.A.; Bryant H.U.
CORPORATE SOURCE: J.A. Dodge, Endocrine Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, United States
SOURCE: Journal of Steroid Biochemistry and Molecular Biology, (1997) 61/1-2 (97-106).
Refs: 30
ISSN: 0960-0760 CODEN: JSBBEZ
PUBLISHER IDENT.: S 0960-0760(97)00008-3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
016 Cancer
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Raloxifene (LY139481 HC1) is a selective **estrogen receptor** modulator (SERM) which blocks the effects of **estrogen** on some tissues, such as the breast and uterus, while mimicking **estrogen** in other tissues, such as bone. To study the origins of this unique pharmacology, we have prepared the major metabolites of raloxifene as chemical probes for examining the **estrogen receptor** function in vitro and in vivo. In human breast cancer **cell** (MCF-7) related assays, these glucuronide conjugates show little affinity for the **estrogen receptor** and are more than two orders of magnitude less potent at inhibiting **cell proliferation** than raloxifene. In non-traditional **estrogen** target tissue, such as bone, these metabolites are less effective than the parent at inhibiting **cytokine**-stimulated bone resorbing activity in rat osteoclasts or producing transforming growth factor beta-3 (TGF-β3). In animal models, tissue distribution studies with radiolabelled metabolite indicate that conversion to raloxifene occurs readily in a variety of tissues including the liver, lung, spleen, kidney, bone and uterus. Differential conversion of metabolite in target organs, such as bone and the uterus, is not observed indicating that the origin of raloxifene's pharmacology does not result from tissue-selective deconjugation of metabolite to parent.

L14 ANSWER 9 OF 15 EMBASE
on STN
ACCESSION NUMBER: 97131078 EMBASE
DOCUMENT NUMBER: 1997131078
TITLE: Role of tumor necrosis factor-alpha in trophoblast function.
AUTHOR: Todt J.C.; Yelian F.D.
CORPORATE SOURCE: Dr. F.D. Yelian, Dept of Obstetrics and Gynecology, CS Mott Ctr Human Growth Development, Wayne State Univ. School of Medicine, 275 East Hancock Avenue, Detroit, MI 48201, United States
SOURCE: Assisted Reproduction Reviews, (1997) 7/1 (17-28).
Refs: 186
ISSN: 1051-2446 CODEN: AEPEEJ
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
010 Obstetrics and Gynecology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Tumor necrosis factor-alpha is a **cytokine** that plays a vital role in inflammation and tissue injury, but also participates in normal **cell** function, including **cell** growth, differentiation, adhesion, and migration. These functions are accomplished via the ability of TNF-α to induce a variety of genes, including cytokines, **cell** adhesion molecules, proteases, major histocompatibility complex proteins, receptors, and transcription factors. Recent studies indicate that along with the phosphatidylcholine-specific phospholipase C pathway by which diacylglycerol is produced, the signal transduction pathway of TNF-α involves the activation of PLA2 by the production of ceramide through the enzyme sphingomyelinase. The resulting production of arachidonic acid leads to the activation of a variety of transcription factors along with the production of prostaglandins and leukotrienes. Tumor necrosis factor-alpha is present at the site of implantation in both mice and humans. Although the source of this TNF-α is debated, its presence at the implantation site, along with the fact that receptors for TNF-α are present on trophoblast cells, suggests that it may play an important role in trophoblast function. This conclusion is also suggested by reports that TNF-α expression in the reproductive tract is controlled by progesterone and **estrogen**. Because TNF-α concentrations are increased in amniotic fluid at term and in preeclampsia (in comparison to first trimester and normal placental tissue respectively) and because TNF **receptor** expression is increased in term placental tissues, it is believed that TNF-α may play an important role specifically during this gestational period. Possible roles include limiting trophoblast invasion of the uterus or modulating maternal immune response to invading trophoblasts. Studies have shown that TNF-α can affect the **proliferation**, differentiation, motility, and hormone synthesis of trophoblasts. Further studies of the role of TNF-α in normal and abnormal trophoblast function may lead to the discovery of treatments of pathologies involving abnormal trophoblast function.

L14 ANSWER 10 OF 15 EMBASE
on STN

ACCESSION NUMBER: 96166130 EMBASE
DOCUMENT NUMBER: 1996166130
TITLE: Leukemia-inhibitory factor stimulates breast, kidney and prostate cancer **cell proliferation** by paracrine and autocrine pathways.
AUTHOR: Kellokumpu-Lehtinen P.; Talpaz M.; Harris D.; Van Q.; Kurzrock R.; Estrov Z.
CORPORATE SOURCE: Department of Bioimmunotherapy, M.D. Anderson Cancer Center, 1515 Holcombe Boulevard,Houston, TX 77030, United States
SOURCE: International Journal of Cancer, (1996) 66/4 (515-519). ISSN: 0020-7136 CODEN: IJCNAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
028 Urology and Nephrology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Leukemia-inhibitory factor (LIF) is an inflammatory **cytokine** with pleiotropic activities. LIF was originally described as a differentiation factor of a murine leukemia **cell** line and was subsequently found to possess a broad spectrum of biological functions. Although LIF has been extensively studied in the hematopoietic system, little is known about its effects in solid tumors. We investigated the role of LIF in breast, kidney and prostate cancers. Using a clonogenic assay, we found that LIF significantly stimulated **proliferation** of 2 **estrogen receptor**-positive breast cancer

cell lines (MCF-7 and T47-D) in a dose-dependent fashion at concentrations ranging from 10 to 200 ng/ml. This effect was observed both in the presence of FCS and under serum- and **estrogen**-free culture conditions, suggesting that the effect of Lip is direct and does not depend on **estrogen** or any other **cytokine**. Neither line produced LIF protein, as assessed by ELISA. In contrast, the **estrogen receptor**-negative breast cancer line MDA MB-231 produced LIF but did not respond to either LIF or its neutralizing antibodies. Similarly, increasing concentrations of Lip did not affect the growth of primary kidney (A-498), metastatic kidney (ACHN) and prostate (DU 145) cancer **cell** lines. These lines produce Lip, however, and antibodies to LIF significantly suppressed their **proliferation** , suggesting that they were maximally stimulated by the endogenously produced **cytokine**. Taken together, our data suggest that Lip acts as either a paracrine or an autocrine growth factor for breast, kidney and prostate cancers.

L14 ANSWER 11 OF 15 EMBASE
on STN

ACCESSION NUMBER: 96027372 EMBASE
DOCUMENT NUMBER: 1996027372
TITLE: Interleukin 6 acts as a paracrine growth factor in human mammary carcinoma cell lines.
AUTHOR: Chiu J.J.; Sgagias M.K.; Cowan K.H.
CORPORATE SOURCE: Medicine Branch, National Cancer Institute, Building 10, 9000 Rockville Pike,Bethesda, MD 20892, United States
SOURCE: Clinical Cancer Research, (1996) 2/1 (215-221).
ISSN: 1078-0432 CODEN: CCREF4
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
016 Cancer
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The effect of interleukin 6 (IL-6) on normal and human mammary carcinoma epithelial cells was studied. IL-6 inhibited the growth of **estrogen receptor**-positive [ER(+)] breast cancer **cell** lines, which underwent apoptosis with prolonged treatment. In contrast, ER(-) breast cancer **cell** lines were resistant to IL-6-mediated growth inhibition. By examining the components of the IL-6 **receptor** (IL-6R) system, we found that ER(+) breast cancer cells expressed predominantly soluble IL-6Rα, whereas the ER(-) breast cancer cells expressed primarily the transmembrane form of the IL-6R, gp130. In addition, detectable levels of IL-6 were secreted into the medium by ER(-) but not ER(+) breast cancer cells. Furthermore, the supernatant obtained from IL-6-secreting, ER(-) cells suppressed the growth of IL-6-sensitive, ER(+) breast cancer cells in a paracrine fashion. Although IL-6 is secreted by ER(-) breast cancer cells, this **cytokine** does not seem to stimulate the **proliferation** of these cells in an autocrine fashion. These studies indicate that IL-6 can regulate the growth of normal and transformed human mammary epithelial cells differentially, and that IL-6 secretion by some ER(-) breast cancer cells can function as a paracrine growth factor, suppressing the growth of ER(+) breast cancer cells in vitro.

L14 ANSWER 12 OF 15 EMBASE
on STN

ACCESSION NUMBER: 95321378 EMBASE
DOCUMENT NUMBER: 1995321378
TITLE: Leukemia inhibitory factor binds to human breast cancer cells and stimulates their proliferation.

AUTHOR: Estrov Z.; Samal B.; Lapushin R.; Kellokumpu-Lehtinen P.; Sahin A.A.; Kurzrock R.; Talpaz M.; Aggarwal B.B.
CORPORATE SOURCE: Department of Bioimmunotherapy, M.D. Anderson Cancer Center, Box 302, 1515 Holcombe Boulevard,Houston, TX 77030, United States
SOURCE: Journal of Interferon and Cytokine Research, (1995) 15/10 (905-913).
ISSN: 1079-9907 CODEN: JICRFJ
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
025 Hematology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Leukemia inhibitory factor (LIF) is a **cytokine** that was originally described as a differentiation factor of a murine myeloid leukemia **cell** line and subsequently found to be an important mediator of embryonic development. Although extensively studied in the hematopoietic system, its effects on solid tumors are generally unknown. In the present study we investigated the role of LIF in human breast cancer cells. Using the reverse transcriptase- polymerase chain reaction, we found that the human breast carcinoma MCF-7 **cell** line expressed the message for both LIF **receptor** and its signal-transducing protein gp130, suggesting that these receptors might be biologically active. Binding studies with radiolabeled LIF demonstrated that MCF-7 cells interacted with this **cytokine**, and the ligand binding was specific and time, dose, and temperature dependent. In addition, a Scatchard analysis of the data revealed a single class of high-affinity (K(d) 0.27 nM) receptors with a density of approximately 430 sites per **cell**. MCF-7 cells exposed to LIF internalized and degraded the ligand. LIF stimulated the growth of MCF-7 as well as other **estrogen**-dependent and independent breast cancer **cell** lines, but the effect on normal breast epithelial lines was less significant. Likewise, it stimulated colony formation by breast cancer cells obtained from five different breast cancer patients in a dose-dependent fashion. These results overall suggest that human breast tumor cells express functional LIF receptors that play a role in breast cancer **cell proliferation**.

L14 ANSWER 13 OF 15 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 93238877 EMBASE
DOCUMENT NUMBER: 1993238877
TITLE: Demonstration of **estrogen** and vitamin D receptors in bone marrow-derived stromal cells: Up-regulation of the **estrogen receptor** by 1,25-dihydroxyvitamin-D3.
AUTHOR: Bellido T.; Girasole G.; Passeri G.; Yu X.-P.; Mocharla H.; Jilka R.L.; Notides A.; Manolagas S.C.
CORPORATE SOURCE: Section of Endocrinology/Metabolism, Veterans Administration Medical Ctr., 1481 West 10th Street,Indianapolis, IN 46202, United States
SOURCE: Endocrinology, (1993) 133/2 (553-562).
ISSN: 0013-7227 CODEN: ENDOAO
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We have shown earlier that 17β-estradiol inhibits **cytokine**

-induced interleukin-6 (IL-6) production by bone marrow-derived stromal cells as well as osteoblasts, two types of cells with a critical influence on osteoclast development, and that ovariectomy causes an IL-6-mediated up-regulation of osteoclastogenesis in mice. Prompted by this, we have searched here for the presence of **estrogen** receptors (ERs) in two murine bone marrow-derived stromal **cell** lines, +/+ LDA11 and MBA 13.2, and the osteoblast-like **cell** line MC3T3-E1. All three **cell** lines exhibited high affinity saturable binding for [125I]17β-estradiol with a dissociation constant of approximately 10-10 M and concentration of binding sites of 260 ± 30, 170 ± 10, and 90 ± 10 sites per **cell**, respectively. In addition, we amplified complementary DNA from the stromal **cell** lines by polymerase chain reaction using oligonucleotide primers flanking the DNA binding domain of the murine uterine ER. The amplified product showed an identical nucleotide sequence to the DNA binding domain of the murine uterine **receptor**. Consistent with the functionality of the ER in stromal cells, and specifically its role in the regulation of IL-6 by 17β-estradiol, we found that the pure **estrogen** antagonist ICI 164,384 completely prevented the effect of 17β-estradiol on IL-6. All three **cell** lines also expressed receptors for 1,25-dihydroxy- vitamin-D3 [1,25(OH)2D3] (dissociation constant, .apprx.10-10 M), with a concentration of binding sites of 490 ± 20, 920 ± 20, and 1110 ± 70 sites per **cell**, respectively. 1,25(OH)2D3 treatment of the stromal cells caused a 2-fold increase in the concentration of ERs and a decrease in **cell proliferation**. These data establish that bone marrow-derived stromal cells express functional **estrogen** as well as vitamin D receptors, which serve to mediate actions of their respective ligands on the biosynthetic activity of these cells and presumably the effects of these two steroid hormones on osteoclastogenesis.

L14 ANSWER 14 OF 15 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 93073646 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1444224
TITLE: Synergistic cytotoxic effects of tumor necrosis factor, interferon-gamma and tamoxifen on breast cancer cell lines.
AUTHOR: Matsuo S; Takano S; Yamashita J; Ogawa M
CORPORATE SOURCE: Department of Surgery II, Kumamoto University Medical School, Japan.
SOURCE: Anticancer research, (1992 Sep-Oct) 12 (5) 1575-9. Journal code: 8102988. ISSN: 0250-7005.
PUB. COUNTRY: Greece
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
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Entered Medline: 19921216

AB The combined effects of recombinant human tumor necrosis factor (TNF), interferon-gamma (IFN) and tamoxifen (TAM) on the **proliferation** of human breast cancer **cell** lines were investigated. In **estrogen receptor** positive MCF-7 cells, relatively resistant to TAM or TNF, cytotoxicity significantly increased in combinations of TNF and IFN, and of a **cytokine** and TAM. The cytotoxicity of TNF increased when cells were pretreated with IFN, but not vice versa. Sequential treatment with IFN following TNF and TAM also exhibited significant antiproliferative effect on both cell lines. The combined or sequential cytokines and TAM treatments are possible modalities to overcome breast cancers unresponsive to endocrine treatment.

L14 ANSWER 15 OF 15 EMBASE
on STN

ACCESSION NUMBER: 91092861 EMBASE
DOCUMENT NUMBER: 1991092861
TITLE: Human endometrial epithelial cell lines for studying steroid and cytokine actions.
AUTHOR: Tabibzadeh S.; Kaffka K.L.; Kilian P.L.; Satyaswaroop P.G.
CORPORATE SOURCE: Department of Pathology, City Hospital Center, Elmhurst, NY 11373, United States
SOURCE: In Vitro Cellular and Developmental Biology - Animal, (1990) 26/12 (1173-1179).
ISSN: 0883-8364 CODEN: ICDBEO
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 001 Anatomy, Anthropology, Embryology and Histology
021 Developmental Biology and Teratology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Recent studies suggest that the **proliferation** and expression of HLA-DR molecules in endometrial epithelium may be regulated by systemic steroids and local cytokines. To test the interacting influences of cytokines and steroids on the expression of HLA-DR and **proliferation** of epithelial cells, an endometrial **cell** model is required that is sensitive to both signals. In this study, we characterize cells of carcinoma **cell** lines of endometrial lineage for their responsiveness to cytokines and steroids. Independently developed for its response to steroid hormones from a well-differentiated adenocarcinoma of human endometrium, EnCa101AE **cell** line is further cloned for the expression of progesterone **receptor**. Immunohistochemical localization using monoclonal antibodies demonstrates that both EnCa101AE **cell** line and cloned ECC1 cells are purely epithelial, as evidenced by the expression of cytokeratin and epithelial membrane antigen, express **estrogen** receptors, and concomitantly exhibit IFN-gamma **receptor**. Experiments using radioiodinated IL-1 reveal that these **cell** lines also possess high affinity receptors for IL-1. As indicated by the induction of HLA-DR molecules, and alterations in morphologic characteristics, these **cell** lines are sensitive to both IFN-gamma and IL-1 action. The class II molecules (HLA-DR, HLA-DP, and HLA-DQ) are differentially induced by IFN-gamma treatment in carcinoma **cell** lines, with HLA-DR being the prevailing induced molecule. IFN-gamma inhibits and estradiol-17β promotes growth of ECC1 cells in a dose- and time-dependent manner. These findings indicate that the interacting effect(s) of the cytokines and steroid hormones on endometrial epithelium may be studied in these unique steroid- and **cytokine**-sensitive epithelial **cell** lines.

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